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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
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EXAMINER	
MAKAR, KIMBERLY A	
ART UNIT	**PAPER NUMBER**
1636	

DATE MAILED: 07/12/2006

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/627,452	PORTNOY ET AL.	
	Examiner	Art Unit	
	Kimberly A. Makar	1636	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *21 March 2006*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *11 and 12* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *11 and 12* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date 7/25/03
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

1. Applicant's election of Group III (claims 11-12) in the reply filed on 03/31/2006 is acknowledged. Because applicant did not distinctly and specifically point out the supposed errors in the restriction requirement, the election has been treated as an election without traverse (MPEP § 818.03(a)).

1. Claims 1-10 are withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to a nonelected invention, there being no allowable generic or linking claim. Election was made **without** traverse in the reply filed on 3/31/2006.

Specification

1. The disclosure is objected to because of the following informalities: Lines 12-15 on page 15 refer to a materials and methods section that is not found in the specification.

Appropriate correction is required.

1. The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

2. Claim 11 is rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a method of activation of B3Z T-cells *in vitro* as a

result of macrophage up-take of *E. coli* bacteria genetically engineered to express a nonsecreted listeriolysin (LLO) operably linked to the tetracycline gene promoter and a second gene expressing chicken ovalbumin (OVA), does not reasonably provide enablement for a method of generating an any immune response to any foreign antigenic agent comprising the administration of any bacteria genetically engineered to encode any nonsecreted cytolysin operably linked to any heterologous promoter and any second gene, one that is other than the cytolysin. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make or practice the invention commensurate in scope with these claims.

3. The test of enablement is whether one skilled in the art could make and use the claimed invention from the disclosures in the specification coupled with information known in the art without undue experimentation (*United States v. Telectronics*, 8 USPQ2d 1217 (Fed. Cir. 1988)). Whether undue experimentation is needed is not based on a single factor but rather is a conclusion reached by weighing many factors. These factors were outlined in *Ex parte Forman*, 230 USPQ 546 (Bd. Pat. App. & Inter., 1986) and again in *In re Wands*, 8 USPQ2d 1400 (Fed. Cir. 1988) and include the following:

4. 1) *The nature of the invention.* Applicants claim 11 is a method of generating an immune response to a foreign antigenic agent consisting of the administration of bacteria genetically engineered to encode a nonsecreted cytolysin operably linked to a heterologous promoter and a second gene, one that is other than the cytolysin. The claimed method reads on the generation of any immune response from the cellular

(presentation of MHC class I antigens vs. MHC class II antigens? *in vitro*) to the systemic level (production of antibodies?) in any organism (i.e. patient) in response to contacting the cell with genetically-engineered bacteria expressing any foreign antigenic gene and a nonsecreted cytolysin for the treatment or prevention of any disease and is not enabled for one skilled in the art to make and/or use the claimed invention in light of the specification.

5. 2) *State of the art.* The invention embodies nascent vaccination technologies utilizing genetically-engineered bacteria to generate an immune response in an organism for the treatment of any disease using any nonsecreted cytolysin and another gene. There are few instances of *in vivo* vaccinations of genetically engineered bacteria expressing a cytolysin and any other gene.

6. 3) *Unpredictability of the art.* The development of vaccinations using attenuated bacteria genetically engineered to produce foreign antigenic agents is an emerging science and unpredictable for the treatment or prevention of many diseases, including, but not limited to, HIV and HCV. Attempts to produce attenuated bacterial vaccines utilizing HIV antigens in cull culture are limited by the high number of immunizations required to elicit an immune response, the relatively low immune response, and the cytotoxic effects of the vaccination (Wu et al, AIDS Research and Human Retroviruses, 1997. 13(14):1187-94 whole article and Burton, PNAS, 1997. 94:10018-10023 whole article). There is nothing specifically noted in the instant specification that identifies how the claimed invention could circumvent or overcome the known problems with

developing a vaccine for a virus such as HIV, and the skilled artisan would have to conduct tremendous amounts of research in order to make and practice the invention.

7. 4) *Number of working examples.* The specification does not provide any working examples of the generation of an immune response from a genetically-engineered bacteria generating an immune/physiological response in an organism from *in vivo* experiments. The working examples are examples of activation of B3T cells in response to the administration of *E. coli* bacteria genetically engineered to produce LLO and OVA to antigen presenting macrophages *in vitro.* The conditions for using cultured cells will vary tremendously from cell type to cell type (media, nutrients, temperature, pH etc.) but will vary even more so compared to the *in vivo* cellular environment where the cells are subjected to alternate conditions (sheer stress in arterial walls vs. low pH in the stomach). Thus there is no evidence in the specification that would enable a skilled artisan to practice the claimed invention for using genetically-engineered bacteria with any gene and any nonsecreted cytolysin for the generation of any immune response in any organism.

8. 5) *Amount of direction or guidance present.* The disclosure provides no specific guidance on how to make or practice the invention *in vivo*. There is little guidance on how to determine acceptable levels of application of the invention *in vivo* to produce a desired immune response for any protective immunity against a specific antigen, how often the application of the invention is required for treatments, or how long expression of an immune response lasts *in vivo*. One skilled in the art would be required to do further experiments in order to use and practice the claimed invention.

9. 6) *Level of skill in the art.* The level of skill is high, but given that the invention relates to areas of molecular biology and medicine that contain many problems yet unresolved, as well as the level of unpredictability, it must be considered that the skilled artisan would have to conduct trial and error in order to attempt to practice the claimed invention.

10. 7) *The breadth of the claims.* The claim is written broadly and read on a method of generating any immune response in a patient using any genetically engineered bacteria that is expressing any nonsecreted cytolysin and any other gene.

11. Given the above analysis of factors which the courts have determined are critical in determining whether a claimed invention is enabled, it must be considered that the skilled artisan would have needed to conduct undue and excessive experimentation in order to practice the claimed invention.

12. It is noted that this Office Action contains rejections of the same claims under 35 USC 112, 1st (enablement) and 35 USC 102 (e). While these rejections may seem contradictory, they are not because each is based upon a different legal analysis, i.e. sufficiency of the disclosure of the instant application to support claims under 35 USC 112, 1st paragraph vs. sufficiency of a prior art disclosure to anticipate or render obvious an embodiment(s) of the claimed invention (See In re Hafner, 161 USPQ 783 (CCPA 1969)).

13. Claim 12 is rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a method of delivering nonsecreted listeriolysin

(LLO) and chicken ovalbumin (OVA) to the cytosol of IC-21 macrophage cells *in vitro* as a result of macrophage up-take of *E. coli* bacteria genetically engineered to express a nonsecreted listeriolysin (LLO) operably linked to the tetracycline gene promoter and a second gene expressing chicken ovalbumin (OVA), does not reasonably provide enablement for a method of generating an any physiological response to any foreign therapeutic agent consisting of the administration of any bacteria genetically engineered to encode any nonsecreted cytolysin operably linked to a any heterologous promoter and any second gene, one that is other than the cytolysin. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make or practice the invention commensurate in scope with these claims.

14. The test of enablement is whether one skilled in the art could make and use the claimed invention from the disclosures in the specification coupled with information known in the art without undue experimentation (*United States v. Telectronics*, 8 USPQ2d 1217 (Fed. Cir. 1988)). Whether undue experimentation is needed is not based on a single factor but rather is a conclusion reached by weighing many factors. These factors were outlined in *Ex parte Forman*, 230 USPQ 546 (Bd. Pat. App. & Inter., 1986) and again in *In re Wands*, 8 USPQ2d 1400 (Fed. Cir. 1988) and include the following:

15. 1) *Nature of the invention.* Applicants claim a method of generating a physiological response to a therapeutic agent consisting of the administration of bacteria genetically engineered to encode a nonsecreted cytolysin operably linked to a heterologous promoter and a second gene, one that is other than the cytolysin. The

claimed method reads on the generation of any physiological response from the cellular (activation of signaling pathways? binding to cellular receptors?) to the systemic level (changes in blood pressure?) in any organism (i.e. patient) in response to contacting the cell with genetically-engineered bacteria expressing any therapeutic gene and a nonsecreted cytolysin for the treatment or prevention of any disease and is not enabled for one skilled in the art to make/practice the claimed invention in light of the specification.

16. 2) *State of the art.* The invention embodies nascent gene therapy technologies utilizing genetically-engineered bacteria to generate a physiological response in an organism for the treatment of any disease using any nonsecreted cytolysin and another gene. The art at the time of this invention shows few *in vivo* examples of successful transmission of foreign genetic or protein material to animal cells using these qualifications, and none for the treatment or prevention of any specific disease in vivo.

17. 3) *Unpredictability of the art.* Gene therapy utilizing any vector system (retroviruses, DNA vaccination, liposomes, bacterial vectors, etc.) is highly unpredictable, with common obstacles including long term versus short term expression, poor transformation efficiency, gene silencing and transient expression of target genes, and successful transformation of the gene does not guarantee robust expression of the protein. One risk factor for using an attenuated bacteria in gene therapy is the potential for those attenuated bacteria to become pathogenic again over time, posing serious health risks to the patient. Grillot-Courvalin et al show that gene transfer efficiency from *E. coli* to mammalian cells varied depending on cell type (page

862, column II, lines 27-29, and Table 2.) Grillot-Courvalin et al suggests that entry into the cell is not necessarily a marker for successful expression of the transgene, and says of a mouse dendritic cell line, "displayed similar numbers of intracellular bacteria after invasion [compared to other cell lines tested], but no expression of the incoming DNA could be detected at 24 h." (Page 864, column II, last paragraph). The author also suggests this discrepancy could be due to degradation of the invading bacteria by the host cell. Grillot-Courvalin also teaches that successful transfer of the DNA into cells did not correlate with the initial number of internalized bacteria (Page 865, Column I, 25-28). Thus gene therapy utilizing attenuated bacterial vectors (i.e. bacteria harboring expression plasmids for the purpose of transmitting foreign genetic or protein material or to a hosts' cell for the generation of a physiological response) is unpredictable in regards to transformation efficiency, cell targeting, and transgene expression.

18. 4) *Number of working examples.* The specification does not provide any working examples of the transmission of foreign genetic or protein material from a genetically-engineered bacteria generating an physiological response in an organism from *in vivo* experiments. The working examples are examples of the transfer of LLO and OVA protein from genetically engineered *E. coli* bacteria to *cultured cells.* The conditions for using cultured cells will vary tremendously from cell type to cell type (media, nutrients, temperature, pH etc.) but will vary even more so to the *in vivo* cellular environment where the cells are subjected to alternate conditions (sheer stress in arterial walls vs. low pH in the stomach). Thus there is no evidence in the specification that would enable a skilled artisan to practice the claimed invention for using genetically-

engineered bacteria with any gene and a nonsecreted cytolysin for the generation of a physiological response in a patient.

19. 5) *Amount of direction or guidance present.* The disclosure provides no specific guidance on how to make or practice the invention *in vivo*. There is little guidance on how to determine acceptable levels of application of the invention *in vivo* to produce a desired therapeutic effect for any specific disease, how often application of the invention is required for treatments, or how long expression of the therapy lasts *in vivo*. One skilled in the art would be required to do further experiments in order to use and practice the claimed invention.

20. 6) *Level of skill in the art.* The level of skill is high, but given that the invention relates to areas of molecular biology and medicine that contain many problems yet unresolved, as well as the level of unpredictability, it must be considered that the skilled artisan would have to conduct trial and error in order to attempt to practice the claimed invention.

21. 7) *The breadth of the claims.* The claim as written is broad and reads on a method of generating an physiological response in a patient using any genetically engineered bacteria that is expressing any nonsecreted cytolysin and any other gene.

22. Given the above analysis of factors which the courts have determined are critical in determining whether a claimed invention is enabled, it must be considered that the skilled artisan would have needed to conduct undue and excessive experimentation in order to practice the claimed invention.

23. It is noted that this Office Action contains rejections of the same claims under 35 USC 112, 1st (enablement) and 35 USC 102 (e). While these rejections may seem contradictory, they are not because each is based upon a different legal analysis, i.e. sufficiency of the disclosure of the instant application to support claims under 35 USC 112, 1st paragraph vs. sufficiency of a prior art disclosure to anticipate or render obvious an embodiment(s) of the claimed invention (See In re Hafner, 161 USPQ 783 (CCPA 1969)).

Claim Rejections - 35 USC § 102

24. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (e) the invention was described in (1) an application for patent, published under section 122(b), by another filed in the United States before the invention by the applicant for patent or (2) a patent granted on an application for patent by another filed in the United States before the invention by the applicant for patent, except that an international application filed under the treaty defined in section 351(a) shall have the effects for purposes of this subsection of an application filed in the United States only if the international application designated the United States and was published under Article 21(2) of such treaty in the English language.

25. Claim 11 is rejected under 35 U.S.C. 102(e) as being anticipated by Darji et al (Cell, 1997. 91:765-775). Claims 11 teach a method of generating an immune response to a foreign antigenic agent consisting of the administration of a bacteria genetically engineered to encode a nonsecreted cytolysin operably linked to a heterologous promoter and a second gene, one that is other than the cytolysin.

26. Darji teaches the oral vaccination of mice using attenuated *Salmonella typhimurium* bacteria containing recombinant vectors expressing a truncated portion (amino acids 26-482) of listeriolysin protein (i.e. a foreign functional nonsecreted cytolysin) and the membrane protein ActA (i.e. a foreign antigenic agent) driven by the heterologous promoter CMV (page 766, column 1, Results Section). Darji teaches that a specific immune response is detected in mice after exposure to bacteria expressing the nonsecreted LLO and the ActA (page 766, column II, lines 17-25, and Figure 1). Thus Darji teaches the claimed invention.

Claim Rejections - 35 USC § 103

27. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

28. Claim 11 is rejected under 35 U.S.C. 103(a) as being anticipated by Powell et al (US Patent No 5,877,159) in view of Darji et al (Journal of Biotechnology, 1995) in further view of Dietrich et al (Nature Biotechnology, 1998) cited in applicants' IDS dated 07/25/03. Claim 11 teaches a method of generating an immune response to a foreign antigenic agent consisting of the administration of a bacteria genetically engineered to encode a nonsecreted cytolysin operably linked to a heterologous promoter and a second gene, one that is other than the cytolysin.

29. Powell et al teaches a method for introducing and expressing genes in animal cells comprising infecting live bacteria that have been engineered to express a variety of agents. Powell teaches this method for the induction of an immune response (i.e. a physiological response) in an animal, more specifically in a human (Claims 15 and 17). Specifically, Powell teaches that a variety of bacteria can be used to generate this immune response including *Shigella spp*, *Listeria spp Rickettsia* and *Escherichia coli* (claim 23). Additional types of bacteria are listed from Column 8 line 49 through column 10 line 46. Powell also teaches that these bacteria are genetically engineered to express the foreign functional cytolysins hemolysin or listeriolysin O (LLO) (column 10 lines 47-56). Additionally, Powell teaches that these cytolysins can be operably linked to an additional agent and transcribed off of a single vector (column 4, lines 29) driven by a heterologous promoter such as SV40, CMV, or RSV (column 15, lines 41-46). Powell also teaches that more that one vector may be used (column 6, lines 65-67, and columns 7, lines 5-8) in order to administer agents. Powell does not teach a nonsecreted foreign functional cytolysin.

30. Darji et al (Journal of Biotechnology, 1995) teaches the hyper-expression of a nonsecreted listeriolysin in the non-pathogenic *Listeria innocua* as a method of producing large quantities of cytoplasmic listeriolysin. Darji teaches that listeriolysin secreted from non-pathogenic bacteria renders them capable of lysis of the phagocytotic vacuole and growth of the bacteria in the cytosol (page 206, lines 10-15).

31. Dietrich et al (Nature Biotechnology, 1998) teaches an attenuated Listeria monocytogenes that are inhibited from intra- and intercellular movement once inside the

cytoplasm and are genetically engineered to carry plasmid DNA encoding GFP and express the phage lysin ply118. The PLY118 lysis protein, "is a late gene product of the Listeria bacteriophage A118 necessary for the release of progeny phage. PLY118 is a wall hydrolyzing enzyme specific for Listeria." (Page 182 Column II, last paragraph, through page 182, column I, first paragraph. Expression of the PLY118 lysin in the attenuated Listeria caused the lysis of the attenuated bacteria, allowing for the release of the GFP construct into the cytoplasm of the infected cell allowing for its transcription. Dietrich also teaches that "the co-expression of the lysin adds to the safety of the attenuated L. monocytogenes...by considerably lowering the number of viable bacteria." (Page 184, column II lines 17-20).

32. One would have been motivated to combine the teaching of Powell on a method for introducing and expressing genes in animal cells comprising infecting live bacteria that have been engineered to express a secreted foreign cytolysin such as LLO and another foreign antigenic agent with the teaching of Darji on the ability to express a foreign nonsecreted listeriolysin in a non-pathogenic bacteria further with the teaching of Dietrich that shows the expression of a lysin inside of a bacteria causes rupture of the bacteria because this increases the safety of the bacterial vector system, as the bacteria are still able to infect a cell and enter a vacuole, but the lysin ruptures the bacteria which allows for (1) a reduced number of viable bacteria entering the cytoplasm and (2) still transferring the foreign antigenic agent. Thus it would have been obvious to combine the teaching of Powell on a method for introducing and expressing genes in animal cells comprising infecting live bacteria that have been engineered to express a

secreted foreign cytolysin such as LLO and another foreign antigenic agent with the teaching of Darji on the ability to express a foreign nonsecreted listeriolysin in a non-pathogenic bacteria further with the teaching of Dietrich that shows the expression of a lysin inside of a bacteria causes rupture of the bacteria because the bacteria would still be able to infect a cell and enter into a vacuole, but the lysin expressed in the bacteria would rupture the bacteria releasing the contents into the vacuole, which would include the foreign antigenic agent and would increase the safety of the system by reducing the number of bacteria able to enter the cytoplasm of the cell. Given the teachings of the prior art and the level of skill of the ordinary skilled artisan at the time the instant invention was made, it must be considered that said ordinary skilled artisan would have had reasonable expectation of success in practicing the claimed invention.

33. Claim 12 is rejected under 35 U.S.C. 103(a) as being anticipated by Powell et al (US Patent No 5,877,159) in view of Darji et al (Journal of Biotechnology, 1995) in further view of Dietrich et al (Nature Biotechnology, 1998) cited in applicants' IDS dated 07/25/03. Claims 12 teach a method of generating an physiological response to a foreign therapeutic agent to an organism (i.e. patient) consisting of the administration of a bacteria genetically engineered to encode a nonsecreted cytolysin operably linked to a heterologous promoter and a second gene, one that is other than the cytolysin.

Powell et al teaches a method for introducing and expressing genes in animal cells comprising infecting live bacteria that have been engineered to express a variety of agents. Powell teaches this method for the induction of a physiological response

(induction of an immune response) in an animal, more specifically in a human (Claims 15 and 17). Specifically, Powell teaches that a variety of bacteria can be used to generate this physiological response including *Shigella spp*, *Listeria spp Rickettsia* and *Escherichia coli* (claim 23). Additional types of bacteria are listed from Column 8 line 49 through column 10 line 46. Powell also teaches that these bacteria are genetically engineered to express the foreign functional cytolysins hemolysin or listeriolysin O (LLO) (column 10 lines 47-56). Additionally, Powell teaches that these cytolysins can be operably linked to an additional agent and transcribed off of a single vector (column 4, lines 29) driven by a heterologous promoter such as SV40, CMV, or RSV (column 15, lines 41-46). Powell also teaches that more that one vector may be used (column 6, lines 65-67, and columns 7, lines 5-8) in order to administer agents. Powell does not teach a nonsecreted foreign functional cytolysin.

34. Darji et al (Journal of Biotechnology, 1995) teaches the hyper-expression of a nonsecreted listeriolysin in the non-pathogenic *Listeria innocua* as a method of producing large quantities of cytoplasmic listeriolysin. Darji teaches that listeriolysin secreted from non-pathogenic bacteria renders them capable of lysis of the phagocytotic vacuole and growth of the bacteria in the cytosol (page 206, lines 10-15).

35. Dietrich et al (Nature Biotechnology, 1998) teaches an attenuated Listeria monocytogenes that are inhibited from intra- and intercellular movement once inside the cytoplasm and are genetically engineered to carry plasmid DNA encoding GFP and express the phage lysin ply118. The PLY118 lysis protein, "is a late gene product of the Listeria bacteriophage A118 necessary for the release of progeny phage. PLY118 is a

wall hydrolyzing enzyme specific for Listeria." (Page 182 Column II, last paragraph, through page 182, column I, first paragraph. Expression of the PLY118 lysin in the attenuated Listeria caused the lysis of the attenuated bacteria, allowing for the release of the GFP construct into the cytoplasm of the infected cell allowing for its transcription. Dietrich also teaches that "the co-expression of the lysin adds to the safety of the attenuated L. monocytogenes...by considerably lowering the number of viable bacteria." (Page 184, column II lines 17-20).

One would have been motivated to combine the teaching of Powell on a method for introducing and expressing genes in animal cells comprising infecting live bacteria that have been engineered to express a secreted foreign cytolysin such as LLO and another foreign therapeutic agent with the teaching of Darji on the ability to express a foreign nonsecreted listeriolysin in a non-pathogenic bacteria further with the teaching of Dietrich that shows the expression of a lysin inside of a bacteria causes rupture of the bacteria because this increases the safety of the bacterial vector system, as the bacteria are still able to infect a cell and enter a vacuole, but the lysin ruptures the bacteria which allows for (1) a reduced number of viable bacteria entering the cytoplasm and (2) still transfers the foreign therapeutic agent. Thus it would have been obvious to combine the teaching of Powell on a method for introducing and expressing genes in animal cells comprising infecting live bacteria that have been engineered to express a secreted foreign cytolysin such as LLO and another foreign therapeutic agent with the teaching of Darji on the ability to express a foreign nonsecreted listeriolysin in a non-pathogenic bacteria further with the teaching of Dietrich that shows the expression of a

lysin inside of a bacteria causes rupture of the bacteria because the bacteria would still be able to infect a cell and enter into a vacuole, but the lysin expressed in the bacteria would rupture the bacteria releasing the contents into the vacuole, which would include the foreign therapeutic agent but would increase the safety of the system by reducing the number of bacteria able to enter the cytoplasm of the cell. Given the teachings of the prior art and the level of skill of the ordinary skilled artisan at the time the instant invention was made, it must be considered that said ordinary skilled artisan would have had reasonable expectation of success in practicing the claimed invention.

Conclusion

No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Kimberly A. Makar, Ph.D. whose telephone number is 571-272-4139. The examiner can normally be reached on 8AM - 4:30 PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Irem Yucel, Ph.D. can be reached on (571) 272-0781. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

KAM/06/15/06

DAVID GUZO
PRIMARY EXAMINER